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**Pitfalls of Internet-accessible diagnostic tests: inadequate performance of a
CE-marked *Chlamydia* test for home use**

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1 **Abstract**

2 **Objectives:** To evaluate the performance of a CE (Conformité Européenne)-marked home
3 test for *Chlamydia trachomatis* (CT) that is available over the Internet.

4 **Methods:** A total of 231 eligible women attending the Social Hygiene Clinic (SHC) or
5 Obstetrics-Gynecology (OB-GYN) Clinic in Iloilo City, Philippines was recruited to an
6 evaluation of the HandiLab-C *Chlamydia* home test (HandiLab-C). One vaginal swab was
7 tested with HandiLab-C on-site and the second at Cambridge UK with two nucleic acid
8 amplification tests (NAAT), the Roche Amplicor and Abbott m2000. Organism load of
9 NAAT-positive swabs was quantified.

10 **Results:** Concordance between the NAAT's was high (kappa agreement: 0.984). Using the
11 Abbott assay as the gold standard, sensitivity and specificity of the Roche assay was 97.4%
12 and 100%, respectively. CT prevalence by Abbott was 8.0% (8/100) in OB-GYN Clinic and
13 23.7% (31/131) at SHC. Sensitivity of HandiLab-C was 12.5% (1/8) and 19.4% (6/31) in
14 OB-GYN and SHC respectively, with specificities of 93.5% (86/92) and 88% (88/100)
15 respectively. Overall positive and negative predictive values of HandiLab-C were 28% and
16 84.5% respectively. No correlation between HandiLab-C performance and organism load
17 (range: 1.3×10^2 - 1.4×10^7 bacteria/swab) was observed.

18 **Conclusions:** The performance of HandiLab-C is unacceptable, with the test yielding more
19 false positive (18/193) than true positive (7/38) results. It remains accessible via the Internet
20 under various brand names and has retained its CE mark. This situation raises serious
21 concerns about the regulation of diagnostic products available via the Internet and the
22 standards of certain Notified Bodies that issue the CE mark.

23

1 **Introduction**

2 Most testing for *Chlamydia trachomatis* (CT) infection is laboratory based, with
3 results provided in 1 to 2 weeks. Rapid tests that can be performed in clinics while the patient
4 remains on-site are commercially available but show variability in both sensitivity and ease
5 of use [1–4]. Given the asymptomatic nature of most CT infections, the availability of a high-
6 performance home test for CT would provide an important additional tool to increase
7 coverage of testing.

8 A CT home-use test is currently marketed via the Internet under various brand names,
9 including HandiLab-C, SureScreen, and SELFCheck. The test is based on the detection of an
10 enzyme (Peptidase 123A) specific to CT [5]. According to the manufacturer (Medical
11 Packaging Corporation, Camarillo, CA, USA), easy to use and has a sensitivity of 98.2% and
12 96.3% for physician or self-collected vaginal swabs, respectively and specificity of 97.9%
13 compared to endocervical samples tested with PCR [6]. The test is CE (Conformitée
14 Européenne) marked as a stamp of quality. The CE marking is based on the In Vitro Medical
15 Device Directive 98/79EC [7] implemented by the European Commission. In vitro medical
16 devices (IVDs) may be CE marked following different routes based on the risk(s) associated
17 with a particular IVD. Chlamydia tests are categorised as Class IIb products, which require
18 the submission of a Technical File to a Notified Body that issues the CE mark. The Technical
19 File gives a summary of the essential requirements according to the IVD Directive including
20 the performance of the test based on the clinical evaluation data and risk analysis on the
21 product. Notified Bodies are designated by the Competent Authority of each member state
22 according to stringent criteria outlined in the directive.

23 A previous study of the HandiLab-C test with 157 women attending a sexual health
24 clinic in Norway found that the test had a sensitivity of 25% (4/16), a positive predictive
25 value (PPV) of 23.5%, and a negative predictive value (NPV) of 85% [8]. In addition, half of
26 the study participants found it difficult to interpret the test results. Its poor performance
27 resulted in the product being taken off pharmacy shelves, first in Denmark and subsequently
28 in Norway [9, 10]. However, the test is still readily available on the Internet with its CE mark
29 in place. Given the large difference between the sensitivity claimed by the manufacturer and
30 that reported in the Norwegian study [8], we evaluated the performance of the HandiLab-C
31 test at sites with a high or low prevalence of CT infection against two nucleic acid

1 amplification test (NAATs): the Abbott M-2000 CT test (Abbott Molecular Division, Abbott
2 Park, IL USA) and Roche Amplicor CT/NG (Roche Molecular Systems, Indianapolis, IN
3 USA). The Abbott m2000 has claims for both self and clinician-collected vaginal swabs as a
4 sample type, showing sensitivity and specificity ranging from 92.5-94.7% and 99-99.8%
5 respectively [11]. The Roche Amplicor CT/NG is not licensed for self-collected vaginal
6 swab, however, published report for this specimen type indicated the sensitivity and
7 specificity to be 90.7-93.3% and 98.8-99.0%, respectively [12].

8 **Methods**

9 **Participants**

10 Consecutive women attending the Social Hygiene Clinic (SHC) or Obstetrics-
11 Gynecology (OB-GYN) Clinic at Western Visayas Medical Center in Iloilo City, Philippines,
12 were recruited during the month of May 2008. Individuals were eligible if they were at least
13 18 years old, had not taken antibiotics during the previous month, had not used a vaginal
14 cream or feminine wash during the previous 24 h, could understand the provided forms, and
15 were willing to participate in the study. Informed consent was obtained from each participant,
16 and the study was approved by the Institutional Research Ethics Committee of the Western
17 Visayas Medical Center.

18 **Specimens and assays**

19 The HandiLab-C is licensed for self-collected vaginal swabs, however, results from
20 self-collected vaginal swab vs. physician-collected swabs was presented by the manufacture
21 on their website [6], showing the latter to be slightly better in performance. To limit sampling
22 variation or errors, HandiLab-C swabs were collected by the clinician according to the
23 manufacturer's. Briefly, HandiLab-C samples were taken by inserting the swab provided by
24 the manufacturer into the vaginal canal approximately 3 to 5 cm and completely rotating the
25 swab 5 times firmly against the vaginal canal.

26 Two vaginal swabs were collected, the first being tested immediately on-site by the
27 clinician with HandiLab-C strictly according to the manufacturer's recommendations [5]. The
28 second swab was collected with the use of Culturette EZ (Becton Dickinson Biosciences, San
29 Jose, CA, USA), frozen at -20°C within 8 hours of collection and shipped to Cambridge UK
30 in dry ice at the end of the study for testing by NAATs. Randomisation of the two swabs for
31 HandiLab-C or NAAT testing would have removed the bias in favour of the former,

1 however, since we were evaluating the performance of the HandiLab-C test, it was decided
2 that the specimens should be tested in the manner recommended by the manufacturer. Given
3 that NAATs are generally acknowledged as far more sensitive than rapid tests, using the
4 second swab for NAAT would have not severely affected the performance of its results.

5 For NAAT testing at Cambridge, frozen swabs were placed overnight in 3 ml of pre-
6 dispensed M4RT medium provided by the manufacturer as part of the Amplicor CT/NG
7 assay kit and tested according to the manufacturer's instructions. Another portion (200 µl)
8 was transferred into the Abbott Specimen Collect Tube and tested by the Abbott m2000
9 RealTime CT assay. Sensitivity, specificity, PPV, and NPV of HandiLab-C were determined
10 using the Abbott m2000 as the gold standard comparator.

11 Organism loads of the specimens found positive by either NAAT were determined
12 according to a previously described method [13, 14] using pCTL12A plasmid as a standard.
13 Eight serial tenfold dilutions bracketing plasmid copy of $10 \cdot 10^8$ per 200 µl was made to
14 generate the standard curve using the decision cycle number provided by the Abbott m2000
15 instrument. The standard curves between runs were highly reproducible ($r^2=0.998$).

16 **Treatment**

17 NAAT results were made available within 3 weeks from the time of sampling.
18 Participants who tested positive for CT by either NAAT were given a single dose (1 g) of
19 azithromycin or, if pregnant, 500 mg of amoxicillin for 7 days according to the guidelines of
20 the Philippine Department of Health [15]. As previous studies have indicated that the results
21 by HandiLab-C were unreliable, treatment decision was based only on NAAT.

22 **Statistical analysis**

23 The 95% confidence interval (CI) was calculated by Wald's method, and differences
24 in test performance were evaluated by the chi-square test. A *P* value of <0.05 was considered
25 statistically significant.

26 **Results**

27 A total of 231 eligible participants was recruited to the study. Most of the SHC
28 participants were commercial sex workers who attended the clinic for a weekly health check,
29 whereas participants at the OB-GYN Clinic were mostly pregnant women attending for
30 antenatal care. The CT positivity rates by the Amplicor assay were 8.0% (8/100) for the OB-
31 GYN Clinic and 22.9% (30/131) for the SHC, consistent with the Amplicor results of a

1 previous study with the same populations at the same sites [15]. Of the total of 231 samples
2 from both sites, 38 samples were concordant positive and 192 concordant negative by both
3 NAAT's. One SHC sample with an organism load of 256 bacteria per swab tested negative
4 by HandiLab-C and Amplicor but gave a repeat positive result with the Abbott assay.
5 Concordance between the NAAT's was high (kappa agreement: 0.984). Using the Abbott
6 assay as the gold standard, sensitivity and specificity of the Roche assay was 97.4% (38/39)
7 and 100%, respectively.

8 On-site testing of the OB/GYN Clinic population ($n=100$) with HandiLab-C detected
9 only one of the eight NAAT-positive samples, giving a sensitivity of 12.5% (95% CI, 0.1-
10 35.4). For the 92 Abbott-negative samples, HandiLab-C yielded 6 false positive results,
11 resulting in a specificity of 93.5% (86/92; 95% CI, 88.4-98.5). Compared with the OB-GYN
12 Clinic population, the sensitivity of HandiLab-C was somewhat higher for the high-risk SHC
13 population ($n=131$), detecting 6 of the 31 Abbott-positive individuals (sensitivity: 19.4%;
14 95% CI: 5.5-33.3, $P=0.70$). However, the specificity was slightly lower at 88% (88/100; 95%
15 CI: 81.6-94.4, $P=0.19$) with 12 false positive results. The sensitivity and specificity of
16 HandiLab-C in the total populations ($n=231$) were 17.9% (7/39; 95% CI: 5.9-30.0) and
17 90.6% (174/192; 95% CI: 86.5-94.8), respectively (Table 1). The overall PPV and NPV were
18 28% (95% CI: 10.4-45.6) and 84.5% (95% CI: 79.5-89.4) respectively. The kappa agreement
19 between HandiLab-C home test and the Abbott or the Roche assay was poor, with the former
20 at 0.100 (95%CI: -0.121 to 0.321) and the latter at 0.105 (95%CI: -0.117 to 0.328).

21 The organism load in the 39 NAAT-positive samples ranged from 1.3×10^2 to $1.4 \times$
22 10^7 bacteria per swab, on the basis of the assumption that each organism harbors seven
23 plasmids [13, 14]. This finding is consistent with that of a previous study with a similar
24 population [4]. There was no apparent correlation between organism load and the HandiLab-
25 C detection rate (Table 2).

26 Discussion

27 A home test based on easy-to-collect vaginal swabs and with a simple testing
28 procedure has the potential to increase uptake of CT testing, especially among young adults
29 who prefer collecting their own specimens and test accessibility via the Internet [16].
30 Consistent with the findings of a previous Norwegian study [8], our results show that the
31 HandiLab-C test has an unacceptably low sensitivity and poor specificity in both low- and

1 high-risk populations. Indeed, HandiLab-C yielded more false positive results than true
2 positive results, making the test an unreliable tool for diagnosis of CT infection. The poor
3 performance of the test was not due to an abnormally low organism load in the tested
4 populations, with the observed load being in the expected range [4].

5 Despite the previous study showing the poor performance of the test [8] and its
6 removal from Danish and Norwegian pharmacies [9, 10], HandiLab-C remains available via
7 the Internet under various brand names and priced between £11.49 and £18.99 and it has
8 retained its CE mark. This situation raises serious concerns about the regulation of diagnostic
9 products available via the Internet and the standards of certain Notified Bodies that issue the
10 CE mark.

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12

1 **Acknowledgments**

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3 Medical Center for their participation and assistance in this study, I. Clarke for providing the
4 pCTL12A plasmid, and J. White and C. Nadala for critical review of the manuscript.

5
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8
9 **Author Contributions**

10 C-E.M. organized study materials, took part in writing and editing the paper, and supervised
11 NAAT assays on matched specimens. F.G.S. was the clinical site coordinator, collected
12 specimens, performed HandiLab-C testing on-site, and organized shipment of specimens
13 from the Philippines to Cambridge. H.J. performed blinded NAAT assays on matched
14 specimens. L.M.M.-T. helped in the design and implementation of the study, was the overall
15 study coordinator, and took primary responsibility for writing the initial draft of the
16 manuscript. H.H.L. was principal investigator of the project, conceptualized the study, and
17 contributed to writing and editing the paper.

18
19 **Competing Interests**

20 C-E.M., L.M.M.-T., and H.H.L. are equity holders of Diagnostics for the Real World Ltd., a
21 spin-off company based on rapid test technologies developed at the University of Cambridge.
22 The University of Cambridge and The Wellcome Trust are also equity holders of the
23 company.

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28

1 **Table 1.** HandiLab-C performance versus NAATs
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	Roche Amplicor		Abbott m2000		Total	
	+	-	+	-		
HandiLab-C	+	7	18	7	18	25
	-	31	175	32	174	206
Total		38	193	39	192	231

4 *The sensitivity and specificity of HandiLab-C compared to Abbott m2000 were 17.9% (7/39; 95%CI: 5.9-30.0) and 90.6%*
 5 *(174/192; 95%CI: 86.5-94.8), respectively. If compared to Roche assay, the sensitivity and specificity of HandiLab-C were*
 6 *thus 18.4% (7/38; 95% CI, 6.1-30.8) and 90.7% (175/193; 95% CI, 79.5-89.4), respectively. The kappa agreement between*
 7 *HandiLab-C home test and the Abbott assay was poor (Kappa: 0.100; 95%CI: -0.121-0.321).*

8
 9
 10 **Table 2.** HandiLab-C and NAATs positivity rate versus organism load
 11

Organism load distribution of the NAAT positive samples (bacteria per swab - $n=39$)							
Methods	10^2-10^3	10^3-10^4	10^4-10^5	10^5-10^6	10^6-10^7	10^7-10^8	Total (%)
Abbott m2000	7/7	7/7	9/9	8/8	6/6	2/2	100
Amplicor	6/7 ^a	7/7	9/9	8/8	6/6	2/2	97.4
HandiLab-C	1/7	1/7	1/9	3/8	1/6	0/2	17.9

12 ^a*One sample with an organism load of 256 bacteria per swab tested negative by Amplicor but yielded a positive result by*
 13 *Abbott. Each standard curve was consistently generated with eight serial tenfold dilutions of the pCTL12A [12] plasmid ($10-$*
 14 *10^8 per 200 μ l) using the decision circle number provide by the m2000 instrument. The reproducibility of the quantitative*
 15 *polymerase chain reaction standard curves was performed in duplicate ($r^2 = 0.998$).*